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BRIEF COMMUNICATION

Cellular and whole plant responses of *Vigna radiata* to NaCl stress

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Abstract

The effect of different NaCl regimes was examined on the growth and ion accumulation in whole plants and callus cultures of *Vigna radiata*. Whole plants grown in sand culture were watered with Hoagland's solution supplemented with 0 - 350 mol m^{-3} of NaCl. Callus cultures were initiated from leaves of 7-d old seedlings of the same seed stock and grown in modified PC-L2 medium containing the same levels of NaCl as in Hoagland's solution. Callus showed the same tolerance to salt as did the whole plant suggesting that *V. radiata* appears to have a mechanism(s) for salt tolerance which operates at the cellular level. Ion analysis of whole plant showed that root sodium concentrations of the tolerant cultivar G-65 was much higher while shoot sodium was much less than those of salt sensitive cultivar ML-1. Callus cultures of cv. G-65 also accumulated higher Na^+ levels. Thus, the greater salt tolerance of cv. G-65 was associated with the control of sodium accumulation at the shoot or cellular level.

The presence of genetic variability for salt tolerance among species of different crops has been documented (*e.g.* Epstein *et al.* 1980) and offers encouragement to develop salt-tolerant plants. Cell culture systems provide a reliable and efficient alternative method for screening, selecting and characterizing salt tolerance at the cellular level (Chandler and Thorpe 1986, Tal 1990). Salt-tolerant cell lines have been isolated from a number of species including a few legumes, *e.g. Medicago sativa* (Croughan *et al.* 1978), *Glycine max* (Jia-Ping *et al.* 1981), *Pisum sativum* (Gosal and Bajaj 1984), *Cicer arietinum* (Pandey and Ganapathy 1984) and *Vigna radiata* (Gosal and

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Bajaj 1984, Kumar and Sharma 1989). Unfortunately, there have been only a few cases where *in vitro* selection resulted in heritable NaCl tolerance expressed at the whole plant level (Nabors *et al.* 1980, Tyagi *et al.* 1981, Vajrabhaya *et al.* 1989, Winicov 1991, Sumaryati *et al.* 1992, Pius *et al.* 1993). In most of the cases, either plants were not regenerated from NaCl-tolerant cell lines or the regenerated plants have not demonstrated increased NaCl tolerance (Smith and McComb 1983). Since selection has been performed at the cellular level, it is important to know, whether salt tolerance in culture is reflected at the whole plant level and whether salt tolerance expressed by intact plants is seen at the level of callus culture. These questions are not adequately resolved in the literature. However, some studies have demonstrated positive correlation between whole plant and cell culture responses for salt tolerance (Tal *et al.* 1978, Orton 1980, Smith and McComb 1981b, Warren *et al.* 1985), while others have shown no correlation between *in vivo* and *in vitro* NaCl tolerance (Smith and McComb 1981a, Hanson 1984, McCoy 1987). The objective of the present study was to determine whether a cellular mechanism for whole plant NaCl tolerance exists in *Vigna radiata.* If such a mechanism exists, then callus culture techniques could be used for screening the germplasm for salt tolerance. This report describes a cellular and whole plant response of *Vigna radiata* to NaCl stress.

Seeds of different cultivars of *Vigna radiata* were procurred from NBPGR, New Delhi and Pulse Research Laboratory, Division of Genetics, IARI, New Delhi.

Ten seeds of each *V. radiata* cultivars K-851, ML-1 and G-65 were germinated in plastic pots, each containing 5 kg of acid washed sand. One week after germination, thinning was done leaving only 4 plants per pot. They were watered with Hoagland's nutrient solution containing either 0, 25, 50, 100, 150, 200, 250, 300 mol m^{-3} NaCl with three replicates per treatment. Extreme care was taken to completely flush all pots at each watering to leach any residual NaCl. The pots were placed in the greenhouse at day and night temperatures of 24 - 27 and 22 - 24 °C, respectively. After 4 weeks, all plants were removed from the pots, the roots were washed with water, excess water was blotted off and fresh mass of each plant was recorded. Plants were then dried at 80 °C for 48 h and dry mass was determined. Four plants from each treatment were selected at random and their shoots and roots were ground separately in a pestle and mortar. 100 mg of well ground tissues were digested with nitric acid as described earlier (Gulati and Jaiwal 1992) and the concentrations of Na^+ and K^+ were determined using an *Elico* flame photometer.

Callus cultures of *Vigna radiata* cv. K-851 were initiated from leaf explants of aseptically grown 7-d-old seedlings. Leaf explants (5 × 5 mm) were transferred aseptically to culture tubes (150 × 25 mm) containing either MS (Murashige and Skoog 1962), B_5 (Gamborg *et al.* 1968), C (MS salts + B_5 vitamins) or PC-L2 (Phillips and Collins 1979) basal media supplemented with NAA (0.5 mg l^{-1}), 2.4-D (0.5 mg l^{-1}) and BAP (1 mg l^{-1}). The media were adjusted to pH 5.8 and solidified with 0.7 % (m/v) agar. The culture tubes were incubated under 16-h photoperiod; irradiance of 80 μmol m^{-2} s^{-1}; temperature 25 ± 2 °C. After establishment, a known amount of callus was subcultured on their respective modified basal media using 5 replicates. After 4 weeks of culture the callus from all replicates was removed and its fresh mass was determined. It was then oven-dried at 80 °C for 48 h prior to

determining its dry mass. The callus growth was maximum at PC-L2 medium and minimum at B_5 (Table 1). Hence PC-L2 medium was used for all other experiments.

Table 1. Growth of leaf explants (after 28 d of culture) of *Vigna radiata* cv. K-851 on different basal media supplemented with 0.5 mg l^{-1} 2,4 D, 0.5 mg l^{-1} NAA and 1.0 mg l^{-1} BAP (mean ± S.E.). Callusing on the all media was 100 %.

Medium	Fresh mass [mg]	Dry mass [mg]
MS	1134 ± 131	106 ± 5
B_5	959 ± 169	85 ± 4
C	1628 ± 33	154 ± 10
PC-L2	1806 ± 40	159 ± 8

The actively growing callus, 250 ± 10 mg, was divided into 10 pieces and inoculated into Petri dishes (100 × 17 mm) containing 20 cm^3 of modified PC-L2 medium supplemented with increasing concentrations of NaCl (0, 25, 50, 100, 150, 200, 250, 300, 350 mol m^{-3}). The Petri dishes were sealed with parafilm and incubated under the same irradiance and temperature as mentioned above. After 4 weeks of culture, callus from Petri dishes, was removed and its fresh and dry masses were determined for each treatment. For each treatment, five replicates were taken and each experiment was repeated twice. Na^+ and K^+ contents of the callus grown on normal and NaCl-containing medium were determined after 4 weeks of culture by the same methods as in whole plant.

The dry mass of whole plants of *Vigna radiata* cvs. K-851 and ML-1 and their corresponding callus cultures decreased gradually with the increasing NaCl concentration (Fig. 1). In cv. G-65, dry mass of whole plant and callus increased under low NaCl concentrations. Similar trends were also observed in fresh mass of the whole plants and calli.

Survival of plants of all the three cultivars was significantly affected by NaCl at concentration higher than 100 mol m^{-3}. At 150 mol m^{-3}, only 40 % of the plants of cvs. ML-1 and K-851 and 50 % of the plants of G-65 survived. However, none of the plants survived of cvs. ML-1 and G-65 at 200 mol m^{-3} NaCl and of cv. K-851 at 300 mol m^{-3}. Similarly, there was almost complete inhibition of callus growth at 300 mol m^{-3} in all three cultivars.

A positive correlation was found between the response of whole plants and calli to NaCl. This led to the suggestion that *V. radiata* appears to have a mechanism(s) of salt tolerance which operates at the cellular level. Similar correlation between the response of the whole plant and callus to salt has also been demonstrated in other glycophytic (Tal *et al.* 1978, Orton 1980, Smith and McComb 1981b) and halophytic (Dains and Gould 1985, Warren *et al.* 1985) species. In some other species, in which no such correlation was found, the organization of the cells in the whole plant has been suggested to be essential for the operation of tolerance mechanism (Smith and McComb 1981a, Hanson 1984, McCoy 1987). The positive correlation found between growth response of whole plant and callus of three cultivars of *V. radiata* to

salt is thus, a pre-requisite for the successful application of callus tissue for salt tolerance screening.

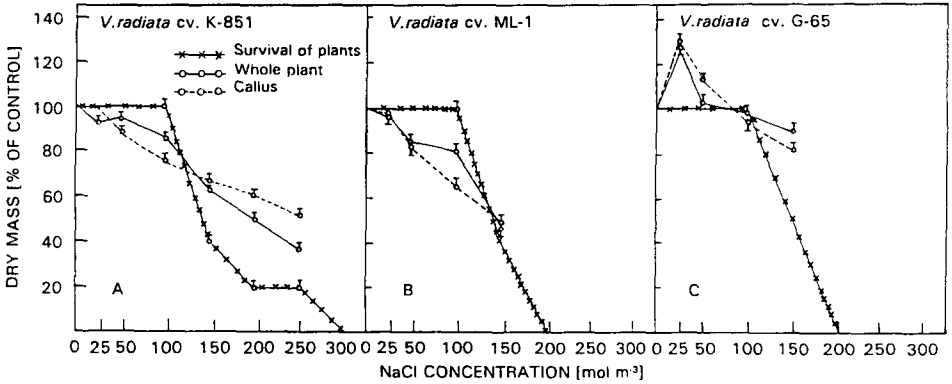

Fig. 1. The effect of NaCl on the survival of plants and growth of whole plant and callus cultures of *Vigna radiata* cvs. K-851 (A), ML-1 (B) and G-65 (C). Vertical bars represent the standard error of the mean.

Table 2. Na^+ and K^+ [μmol g^{-1}(d.m.)] and K^+/Na^+ ratio in shoots and roots of NaCl-treated plants of three cultivars of *Vigna radiata* after 28 d of culture (mean of 5 replicates ± S.E.).

Cultivar	NaCl [mol m^{-3}]	Shoot			Root		
		Na^+	K^+	K^+/Na^+	Na^+	K^+	K^+/Na^+
G-65	0	2.1 ± 0.1	18.5 ± 1.9	0.80	15.6 ± 1.0	29.4 ± 2.5	1.88
	25	3.0 ± 0.1	20.7 ± 0.2	6.82	19.5 ± 1.1	27.1 ± 1.0	1.39
	50	8.0 ± 0.4	19.2 ± 0.1	2.39	29.3 ± 1.0	26.2 ± 1.9	0.89
	100	13.4 ± 0.4	18.2 ± 0.2	1.35	26.0 ± 1.2	20.5 ± 2.0	0.78
ML-1	0	2.1 ± 0.1	19.2 ± 1.0	8.84	4.3 ± 0.3	32.0 ± 3.8	7.27
	25	4.3 ± 0.2	21.7 ± 2.0	5.01	19.9 ± 1.7	27.8 ± 1.7	1.39
	50	14.1 ± 1.0	21.0 ± 2.1	1.48	31.5 ± 1.0	17.3 ± 0.6	0.55
	100	22.6 ± 2.0	20.5 ± 1.2	0.90	21.7 ± 1.7	14.1 ± 0.5	0.65
K-851	0	2.1 ± 0.1	14.7 ± 0.6	6.78	22.1 ± 0.4	33.9 ± 1.9	1.53
	25	3.6 ± 0.3	21.4 ± 0.3	5.79	26.5 ± 1.7	36.7 ± 0.8	1.38
	50	5.4 ± 1.0	17.3 ± 0.6	3.18	31.5 ± 1.0	35.8 ± 2.0	1.14
	100	8.0 ± 0.6	22.0 ± 1.0	2.74	35.3 ± 3.0	35.2 ± 2.5	0.99

The K^+/Na^+ ratio in shoot and root of whole plants of cvs. G-65, K-851 and ML-1 decreased with increasing salt levels (Table 2). This decrease in K^+/Na^+ ratio was due to increase in Na^+ content of both the tissues. The K^+/Na^+ ratio was much higher in the shoot than the root suggesting greater K^+ content in shoot. However, in the root, K^+/Na^+ ratio were much higher in cvs. G-65 and K-851 than ML-1, suggesting that the former two are more tolerant than the latter. Similarly, higher K^+/Na^+ ratios in roots of salt-tolerant than of salt-sensitive cultivars were also found in *Zea mays* (Hajibagheri *et al.* 1987) and *Cajanus cajan* (Subba Rao *et al.* 1990). In the present

study, cv. ML-1 exhibited greater increase in Na^+ content in shoot than G-65, at identical NaCl concentrations. Though, uptake of Na^+ was not prevented in cv. G-65, but apparently an inhibition of diffusion or transport of Na^+ into xylem sap possibily by cellular or subcellular compartmentation results in low levels of Na^+ in the shoot. Similar low Na^+ accumulation in the shoot was found in salt-tolerant genotypes of many crop plants (Subba Rao *et al.* 1990, Schachtman and Munns 1992). However, K^+ content in the shoot of G-65 was almost similar to that of ML-1. These results suggest that salt tolerance is expressed at the tissue level and are compatible with the model where Na^+ is compartmentalized in G-65 root tissue, thus restricting its translocation to the shoot and interference with K^+.

In calli *of V. radiata* cvs. G-65 and ML-1 accumulation of Na^+ increased, while that of K^+ decreased with increasing NaCl concentration (Table 3). Cultivar G-65 showed high Na^+ and low K^+ content compared to cv. ML-1. The increase in Na^+ content and decrease in K^+ content of cv. G-65 may be due to low efficiency of Na^+ exclusion and K^+ uptake in this cultivar. It is suggested that a better osmotic adjustment by higher Na^+ concentration and at the same time preventing the cells from toxic effects is responsible, at least in part, for the superior performance of this cultivar under salinity stress. These results are in accordance with those for

Table 3. Na^+ and K^+ [μmol g^{-1}(d.m.)] and K^+ /Na^+ ratio in NaCl-treated callus of *Vigna radiata* after 28 d of culture (mean of 5 replicates ± S.E.).

Cultivar	NaCl [mol m^{-3}]	Na^+	K^+	K^+ /Na^+
G-65	0	15.2 ± 0.0	117.9 ± 0.0	7.75
	100	61.9 ± 5.4	52.5 ± 8.9	0.85
	200	74.9 ± 1.0	32.6 ± 5.7	0.43
	300	92.3 ± 3.2	25.6 ± 0.0	0.27
ML-1	0	9.7 ± 1.1	98.3 ± 1.6	10.10
	100	42.6 ± 0.8	42.9 ± 0.6	1.00
	200	64.1 ± 1.1	42.1 ± 4.4	0.65
	300	75.8 ± 3.8	25.2 ± 0.8	0.33

Lycopersicon species (Tal *et al.* 1978) and *Medicago sativa* (Croughan *et al.* 1978). Our other studies on selection and characterisation of NaCl-tolerant cell line of *V. radiata* cv. K-851 indicate that NaCl-tolerant line not only accumulated more Na^+ but also maintain higher levels of K^+ than sensitive lines. It appears that salt selected cells do not exclude salt as the mechanism of tolerance but it utilizes uptake of these ions for osmotic adjustment and enhancing turgor like the most tolerant cv. G-65 (Gulati 1992). Since, the cultured cells have different hormonal, osmotic and nutritional environment from cells in whole plants; therefore it is suggested that the emphasis should be on the comparison of the mechanism of salt tolerance in whole plants and in isolated cells under identical environmental conditions. The cells of the most tolerant cultivar G-65 and the salt-selected calli of *V. radiata* cv. K-851 have accumulated more Na^+ ions. Therefore, selection of cultured cells may be more

productive if focussed on specific cell based physiological traits such as Na^+ accumulation and turgor regulation.

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